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(d) growing the fused cells from step (c) in a selection medium which selects for cells with mitochondrial DNA; and

(e) selecting cells from step (d) which contain a nucleus which originated from the cells of the primary culture, so as to prepare the human immortalized cell line.

REMARKS

Claims 1, 3-5, 8-10 and 12 are pending in the subject application. Applicant has hereinabove amended the specification and claims 1 and 8. Accordingly, upon entry of this Amendment, claims 1, 3-5, 8-10 and 12 will still be pending and under examination.

In making these amendments, applicant neither concedes the correctness of the Examiner's rejections in the November 20, 2002 Office Action, nor abandons the right to pursue in a continuing application embodiments of the instant invention no longer claimed in this application. Applicant maintains that these amendments to the specification and claims do not raise any issue of new matter, and that these claims are fully supported by the specification as originally filed. Accordingly, applicant respectfully requests that this amendment be entered.

Pursuant to the requirements of 37 C.F.R. §1.121(b), applicant attaches hereto as **EXHIBIT A** a marked-up

version of the amended paragraph showing the changes relative to the previous version thereof.

Pursuant to the requirements of 37 C.F.R. §1.121(c), applicant attaches hereto as **EXHIBIT B** a marked-up version of the amended claims showing the changes relative to the previous version thereof.

In view of the arguments set forth below, applicant maintains that the Examiner's objections and rejections made in the November 20, 2002 Office Action have been overcome, and respectfully requests that the Examiner reconsider and withdraw same.

Formalities

The Examiner objected to the specification because it identifies DMEM F12 as "Dubeco's minor essential medium" at page 17, line 9.

In response, applicant has hereinabove amended the specification to correct this informality.

Claim Objections

The Examiner objected to claims 8-10 and 12 since claim 8 contains a minor grammatical error.

In response, applicant has hereinabove amended claim 8 to correct the grammatical error.

Claim Rejections Under 35 U.S.C. §112, First Paragraph

The Examiner rejected claims 8-10 and 12 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Specifically, the Examiner based his rejections, in relevant part, on the assertion that "depletion of mitochondria does not affect the ability to perform glycolysis."

In response, applicant respectfully traverses the Examiner's rejection. Without conceding the correctness of the Examiner's position, applicant notes that neither amended claim 8 nor claims 9, 10 and 12, recite the term "glycolysis." Thus, the Examiner's remarks are inapposite to the claims as amended, and the claimed method is enabled.

In view of the above remarks, applicant maintains that claims 8-10 and 12 satisfy the requirements of 35 U.S.C. §112, first paragraph.

Claim Rejections under 35 U.S.C. §102(b)

The Examiner rejected claims 1 and 3-5 under 35 U.S.C. §102(b) as allegedly anticipated by Wang et al. (In Vitro Cellular and Developmental Biology 27(1): 63-74, 1/1991).

In response, applicant respectfully traverses the Examiner's rejection.

Claim 1, as amended, provides an immortalized human cardiomyocyte cell line. The claimed cell line is produced by a method comprising the step of fusing a post-mitotic primary non-immortalized human cardiomyocyte with a human fibroblast. The fibroblast has the features of having been treated with ethidium bromide, comprising a replicable vector which confers immortality on a cell comprising same, and being free of mitochondrial DNA. Claims 3-5 provide specific embodiments of the cell line of claim 1.

For Wang et al. to anticipate the cell line of claims 1 and 3-5, it would have to teach each and every feature thereof. It fails to do this, and the Examiner has not established any teaching to the contrary. Accordingly, Wang et al. fails to anticipate the cell line of claims 1 and 3-5.

In view of the above remarks, applicant maintains that claims 1 and 3-5 satisfy the requirements of 35 U.S.C. §102(b).

Summary

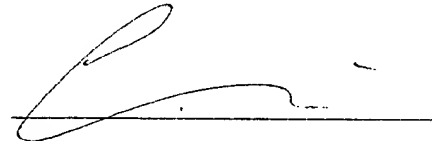
Applicant maintains that the claims pending are in condition for allowance. Accordingly, allowance is respectfully requested.

If a telephone conference would be of assistance in advancing prosecution of the subject application, applicant's undersigned attorney invites the Examiner to telephone him at the number provided below.

Applicant: Mercy M. Davidson
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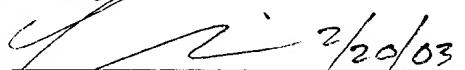
No fee is deemed necessary in connection with the filing of this Amendment. However, if a fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,



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I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231

 7/20/03
Alan J. Morrison
Reg. No. 37,399

MARKED-UP VERSION OF THE AMENDED PARAGRAPH

Additions to the text are double-underlined; deletions are bracketed in square brackets.

In the Specification

Please delete page 16, line 33 to page 17, line 13, and insert the following replacement paragraph:

--Since the primary cultures stopped dividing, an indirect method was used to transfer the SV-40 gene in order to immortalize the cardiomyocytes. Used was a fibroblast cell line (DWFb1) transfected with a plasmid pNRS1, an SV-40 based mammalian vector, and depleted of its mitochondrial DNA (mtDNA) by treatment with ethidium bromide. DWFb1 was entirely dependent on glycolysis for energy requirements and was auxotrophic to uridine and pyruvate. A few days after the final complement fixation step, DWFb1 cells were layered on the cardiomyocytes and allowed to attach for 4 hours at 37°C (fusing the ρ^0 cells with primary cells in culture). The cells were fused with a 50% polyethylene glycol (PEG) solution for one minute, excess PEG was removed, the cells were gently rinsed in 10% dimethyl sulfoxide (DMSO) in culture medium, and subsequently grown under selection in uridine-free [Dubecco's minor essential medium] Dulbecco's Modified Eagle's Medium (DMEM) F-12 medium supplemented with 12.5% dialysed Fetal Bovine serum (FBS). This selection will eliminate the mtDNA-less cells (ρ^0) cells that have not fused with the cardiomyocytes (selecting cells for a cell line).--

MARKED-UP VERSION OF THE AMENDED CLAIMS

Additions to the text are double-underlined; deletions are bracketed in square brackets.

Please amend claims 1 and 8 as follows:

1. (Amended) An immortalized human cardiomyocyte cell line wherein the cell line is produced by a method comprising the step of fusing a post-mitotic primary non-immortalized human cardiomyocyte with a human fibroblast, the fibroblast

- (a) having been treated with ethidium bromide,

- (b) comprising a replicable vector which confers immortality on a cell comprising same, and

- (c) being free of mitochondrial DNA.

8. (Amended) A method for preparing a human immortalized cell line derived from a post-mitotic primary cell culture which comprises:

- (a) providing a cell culture of human primary post-mitotic cells[,];

- (b) providing a human fibroblast cell line which[:]

- (i) has been transfected with a replicable nucleic acid vector

which immortalizes the
fibroblast cell line, and

- (ii) has been depleted of its
mitochondrial DNA [thereby
rendering the fibroblast cell
line subject to growth selection
due incapacity to perform
glycolysis];
- (c) co-culturing the human fibroblast
cell line of step (b) with the cell
culture of step (a) under
appropriate conditions so that cell
fusion occurs; [and]
- (d) growing the fused cells from step
(c) in a selection medium which
selects for cells with mitochondrial
DNA[,]; and
- (e) selecting cells from step (d) which
contain a nucleus which originated
from the cells of the primary
culture, so as to prepare the human
immortalized cell line.